

## REMARKS

1. Present Status of Patent Application

Upon entry of the newly added claims in this supplemental response, claims 8, 10-14, 16-18 and 23-39 remain pending in the present application. More specifically, claims 34-39 are newly added herein. It is believed that the foregoing amendments and additions add no new matter to the present application.

2. Response to Notice of Non-Compliant Amendment Under 37 C.F.R. 1.121

This is a full and timely response to the outstanding Notice of Non-Compliant Amendment under 37 C.F.R. 1.121 mailed October 25, 2002 ("Notice"). The Notice stated that a clean version of the replacement paragraphs are required under 37 C.F.R. 1.121(b)(1)(ii). In particular, the Notice stated that amendments must have complete paragraphs. Applicants herein comply with the requirement of 37 C.F.R. 1.121 by providing the entire paragraph that was amended, beginning on page 40, line 21 – page 43, line 5. Applicants respectfully request that the non-compliant amendment now be entered.

3. Supplemental Reply under 37 C.F.R. 1.111(a)(2)

As a Supplemental Reply under 37 C.F.R. 1.111(a)(2), further in response to the nonfinal Office Action mailed April 10, 2002, please add new claims 34-39 above. New claims 34-39 have been newly added to further define and/or clarify the scope of the invention. New claims 34-39 are believed to be allowable over the cited prior art, for at least the same reasons that claim 8 is allowable over the cited prior art, as detailed in Applicants' response mailed on October 10, 2002. Applicants respectfully request that newly added claims 34-39 be considered in the response to the nonfinal Office Action mailed April 10, 2002.



### CONCLUSION

Applicants respectfully submit that the specification has been amended in compliance with 37 CFR 1.121, and new claims have been added pursuant to 37 C.F.R. 1.111(a)(2). Applicants respectfully request that the Examiner pass this case to issuance. If, in the opinion of the Examiner, a telephone conference would expedite the examination of this matter, the Examiner is invited to call the undersigned attorney at (770) 933-9500.

No fee is believed to be due in connection with this response. If, however, any fee is deemed to be payable, you are hereby authorized to charge any such fee to Deposit Account No. 20-0778.

Respectfully submitted,

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**ANNOTATED VERSION OF MODIFIED SPECIFICATION**  
**TO SHOW CHANGES MADE**

The following is a marked up version of the amended specification. Amend the following page/paragraph text by adding the language that is underlined (“\_\_\_”) and by deleting the language that is enclosed within brackets (“[ ]”):

Page 43, first paragraph, lines 1-5:

From a single specimen of sea anemone *Actinia equina* L., total RNA was isolated by disrupting the tissue in a liquid nitrogen. Two grams of ground, deeply frozen tissue was transferred into 20 ml of guanidinium thiocyanate solution (5.5 M GTC, 0.5 M EDTA, 0.2 M  $\beta$ -mercapto-ethanol) and homogenized in an electrical mixer (16,000 rpm, 4x 30 sec.). Subsequently, the solution was centrifuged at 6000 X g, 20 min. at 15°C. 10 ml of clear supernatant was transferred to 10 ml of Cesium TFA solution ( $\rho$ =1.5 mg/ml), supplemented with 2.5 ml of 0.5 M EDTA, pH=8.0. Sample was centrifuged for 24h at 125,000 X g, 15°C. The supernatant was removed and total RNA (pellet) was dissolved in 1 ml of proteinase K solution (0.5 mg/ml). After incubating the solution at 50°C, 1/2h, total RNA was precipitated by 1/10 volume of 3M KOAc, pH=5.2 and 2.5 vol. of 96% ethanol and the mixture was placed overnight in the freezer at -20°C. Total RNA was pelleted at 8000xg and the pellet was dissolved in 2 ml of TE buffer. 1 ml of dissolved sample was heated at 65°C for 5 min, cooled on ice and subsequently, 0.2 ml of TE buffer, supplemented with 3M NaCl was added. Whole sample was applied to the top of the oligo (dT) – cellulose bed in the column. The cellulose was washed three times with the TE buffer, supplemented with 0.5 M NaCl and 0.1 M NaCl, respectively. Poly(A)+ RNA was eluted with 1 ml (divided into 4 aliquots of 0.25 ml) of TE buffer, prewarmed to 65°C. One third of the sample (approx. 1  $\mu$ g) was used for a synthesis of cDNA according to manufacturer’s procedure (Amersham). First strand cDNA synthesis was performed using 11  $\mu$ l of mRNA solution, 4  $\mu$ l 5 X reaction buffer, 1  $\mu$ l Na-pyrophosphate solution, 1  $\mu$ l human placental ribonuclease inhibitor (5U/ $\mu$ l), 2  $\mu$ l of dNTP mix solution and oligo dT primer solution (1  $\mu$ l). After addition of 2  $\mu$ l (10 U/ $\mu$ l) of reverse transcriptase, a mixture was incubated at 42°C for 40 minutes. Second strand cDNA was synthesized by adding the following components: 37.5  $\mu$ l of second strand reaction buffer, 7  $\mu$ l of E. coli DNA polymerase I (4U/ $\mu$ l) and 1  $\mu$ l of E.

coli ribonuclease H (1U/ $\mu$ l). The reaction mix was incubated sequentially at 12°C for 60 minutes and 22°C for 60 minutes. After heat denaturation (5 minutes at 70°C), 0.5  $\mu$ l of T4 DNA polymerase (2U/ $\mu$ l) was added and the reaction mix was incubated at 37°C for 10 minutes. 2.5  $\mu$ l (250 pmoles) of EcoRI-adaptors were ligated to 1  $\mu$ g of cDNA, using 2  $\mu$ l of T4 DNA ligase (4U/ $\mu$ l) in 20  $\mu$ l of ligation mixture. After 8h at 16°C, the ligation mixture was subjected to column purification/size fractionation of adaptor-linked cDNA, using spun columns and TE buffer. Collected fractions of purified cDNA were phosphorylated by T4 polynucleotide kinase (8U/ $\mu$ l) and cDNA was ligated into dephosphorylated  $\lambda$ gt11 bacteriophage arms using T4 DNA ligase (40U/ $\mu$ g DNA). Finally, whole ligation mixture was *in vitro* packaged using packaging extract from the same manufacturer (Amersham). A portion of  $\lambda$ gt11 cDNA library ( $10^5$  pfu) was used for the infection of Y1090 *E. coli* cells and mixture was plated onto LB agar plates. Plaques were blotted onto nitrocellulose membranes. Membranes were rinsed in Tris-buffered saline with 0.01% Tween-20 (TBST) 3 times and subsequently in blocking solution (20% (v/v) fetal serum in TBST). After washing the membranes in TBST, the first antibody (rabbit anti equistatin IgG) in TBST was added and membranes were treated in solution overnight at 4°C. Afterthat, membranes were washed three times with TBST, and treated with second antibody (goat anti rabbit IgG.–horse raddish peroxidase). After final washing in TBST, a visualization with diaminobenzidine as a substrate was performed. Three positive clones were isolated from agar plates and after re-plating, phages were eluted from the surface of the agar plates with TE buffer (5 ml per plate). Phage DNA was isolated using Wizard Lambda Preps DNA isolation kit (Promega) according to the manufacturer's procedure. After restriction analysis with 2  $\mu$ l of EcoRI restriction enzyme (10U/ $\mu$ l) per 3  $\mu$ g of  $\lambda$ DNA and size fractionation on 1% agarose gel, cDNA inserts were excised, purified with glass milk and subcloned into *Eco*RI cloning site of pUC19 plasmid. Whole ligation mixture (10  $\mu$ l of each sample) was transformed into DH5 $\alpha$  *E. coli* cells by incubating the 100  $\mu$ l of highly competent cells (O.D.<sub>550</sub>=0.6) and 10  $\mu$ l of ligation mixture in a water bath (42°C) for 45 seconds. After addition of LB medium (900  $\mu$ l) and 1h incubation (37°C, 250 rpm), bacterial mixture was plated onto LBA plates, supplemented with X-gal and IPTG and after overnight incubation (37°C), white colonies were transferred into 5 ml of LB medium and incubated for an additional 16 hours (37°C, 250 rpm). Plasmids were isolated using Wizard Plasmid Purification System (Promega) according to manufacturer's instructions and analysed by nucleotide sequencing using T7 DNA polymerase (T7 sequencing kit, Pharmacia) and [<sup>35</sup>S]dATP $\alpha$ S (Amersham). Sequencing of selected cDNA

clones resulted in the full length cDNA clone given in Figure 1. The DNA sequence of FIG. 1 corresponds to SEQ ID NO:1. The protein sequence of FIG. 1 corresponds to SEQ ID NO: 2.